Journal of Pioneering Medical Sciences
Received: March 11, 2025 | Accepted: April 13, 2025 | Published: August 05, 2025
Volume 14, Issue 07, Pages 127-131

DOI https://doi.org/10.47310/jpms2025140720

Evaluation of Histological Changes Resulting from the Effect of the Drug Pregnyl on the Histological Structure of The Lungs in the White Mouse Mus Musculus

Ashwak T. Humadi[1*], Ibtisam Khalaf Abd ali[2] and Thekra Atta Ibrahim[3]
[1-3]Department of Biology, College of Education for Pure Science, Diyala University, Diyala, Iraq. 2College of Nursing, University of Baghdad, Iraq

Author Designation: [1]Associate Lecturer, [2]Associate Professor, [3]Professor

*Corresponding author: Ashwak T. Hamadi (e-mail: ashwaqaljubory@uodiyala.edu.iq).

©2025 the Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0

Abstract The present investigation aimed to identify the effect of the drug pregnyl on the histological structure of the lungs in male Swiss mice (Mus musculus). The study included the use of 14 mice divided into two groups, with 14 mice in each group, and the animals of each group were placed in a separate cage. The first group, which is the control group, its animals were treated with distilled water only for 30 days, while the mice of the second group, which is the experimental group, were injected by concentration of 0.2 mg of pregnyl/kg of body weight intraperitoneally, for 30 days too. On the last day, the animals were sacrificed, their lungs were extirpation, and their tissue sections were prepared. The results obtained from the histological study showed that all animals treated with pregnyl exhibited pathological histological changes in the lungs, represented by degeneration and necrosis of the cells lining some bronchi and the separation of some cells from the epithelial layer. It was also noted that there was a gap between the epithelial tissue and the submucosal layer. The results of the study showed the occurrence of edema between the alveoli and alveolar sacs and an increase in the thickness of the wall between the alveoli. On the other hand, congestion and bleeding occurred in the blood vessels, and cellular infiltration was observed between the alveoli and alveolar sacs. It was also noted that there was a break in the wall of the alveoli and alveolar sacs and their accumulation inside the alveolar cavity. It was also noted that there was a collection of inflammatory cells and blood congestion between the alveoli and alveolar sacs. It was also noted that there was a rupture between the alveoli and the occurrence of blood bleeding. The blood vessel was distorted and congested with the appearance of a blood clot inside the blood vessel, in addition to the occurrence of fibrosis and infiltration of inflammatory cells around the vessel. In addition, active transport vesicles inside the vessels appeared, indicating a high activity of the blood vessel lining.

Key Words Histology, Lung Tissue, Mus Musculus, Pregnyl, Reproductive Hormones, White Mouse

INTRODUCTION

Steroids are a group of lipophilic, low molecular weight compounds all derived from cholesterol, so different classes of steroids are very similar to each other because they all have the same basic structure [1]. Slight variation in this structure in terms of the nature of the attached groups, the location of the groups, and the composition of the activator core leads to the formation of different types of activators, in addition to the fact that small modifications in the molecular structure of activators can produce noticeable differences in their biological activities [2].

Studies of steroids began in the early 19th century with unsaponifiable substances (i.e., remaining undissolved after heating with an excess of alkali), most of which were cholesterols found in animal fats and acids that could be obtained from bile [3]. This early work led to the isolation of cholesterol and some bile acids, their purification, and the identification of some important features of their chemical structure. However, the complex structure of polycyclic steroids was not recognized until the beginning of the 20th century, after the unification of chemical theory and the development of chemical techniques by which these molecules could be divided [4].

Other studies have determined the composition of cholesterol from related sterols, ergosterol, and bile acids, particularly studies by the German chemists Adolf Winduous and Heinrich Wieland [5]. The family of steroids includes sterols, bile acids, a number of hormones

(gonadotropic and adrenal cortical hormones), some hydrocarbons, and pregnyl [6]. Pregnyl is a polypeptide steroid drug consisting of 237 amino acids with a molecular weight (MW) of approximately 37 kDa. It is produced in the human placenta and obtained from the urine of pregnant women and is a purified preparation consisting of an alpha and beta subunit [7].

The alpha subunit is essentially identical to the alpha subunits of human pituitary gonadotropin, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as the alpha subunit of human thyroid-stimulating hormone (TSH) [8]. The beta subunits of these hormones differ in their amino acid sequence. Percnyl is primarily prescribed to men to increase testosterone and sex cells [9]. The action of pericardium is almost identical to that of LH in the pituitary gland, in that it stimulates the production of gonadal steroid hormones by stimulating the interstitial cells (Leydig cells) of the testis to produce androgens and the corpus luteum of the ovary to produce progesterone [10]. Androgen stimulation in males leads to the development of secondary sexual characteristics and may induce testicular descent when there is no anatomical impediment to descent [11].

The most well-known benefit of Pregnyl injections is the stimulation of testosterone production in males. The anabolic effect of increased endogenous androgen production produces physical advantages in male athletes, particularly in strength sports, and as such is a means of indirect androgenic doping [12]. However, this use is not without side effects. The reported side effects of this drug in males are hypersensitivity, whether local or systemic, irritation, insomnia, depression, headache, general disorders and injection site conditions. While in females, the side effects were vascular disorders, respiratory, thoracic and mediastinal disorders, gastrointestinal disorders, and reproductive system disorders (breast) [13]. Given the great importance of the lungs due to their locations and the multiple functions they perform, and the lack of most previous research on the tissue effects caused by drugs and the lack of recorded studies on the effect of this drug on this active organ, this study aimed to investigate the effect of the drug Pregnyl on the tissue structure of the lungs in adult white mice after injection for 30 days.

MATERIALS AND METHODS

Dose preparation: The desired dose of pregnyl was prepared according to Equation (1)

$$\frac{x}{D} = \frac{W_{mouse}}{1000} \quad (1)$$

Where, x: mass of pregnyl drug that injected to mouse in experiment (g), D: desired dose of pregnyl, which is 0.2 mg of pregnyl/kg of mouse' body weight, and W_{mouse}: is the weight of mouse used in experiment.

RESULTS

Animals Used in Experiments and Histological Studies

In this study, 14 male white mice were used, which were obtained from the animal house of the College of Veterinary Medicine / University of Baghdad, and their average weights ranged between 25-30 g. These mice were divided randomly into two groups, the details of which were as follows: the first group is the control group of 7 mice and the second group is the test group of 7 mice, which were injected with pregnyl at a concentration of 0.2 mg/ kg of body weight daily for 30 days. On the last day, the mice were anesthetized by chloroform, then the animals were dissected and the lungs were removed carefully from their site. After that, the samples were fixed in formalin solution for 24 hours, then washed with tap water and transferred to 70% alcohol for preservation. The tissue sections were prepared according to method described in [14], where the samples were passed through an ascending series of ethyl alcohol, then placed in xylene solution for clarification, and embedded in paraffin wax. The prepared wax molds were cut using a rotary microtome with a thickness of 7 microns. The obtained glass sections were stained using Harris Haemotoxylin and Eosin (H&E) stain according to the method followed by (Bancroft and Gamble ,[15] The stained-glass sections were loaded with Canada Balsam, then the samples were examined and photographed using a light microscope equipped by a digital camera

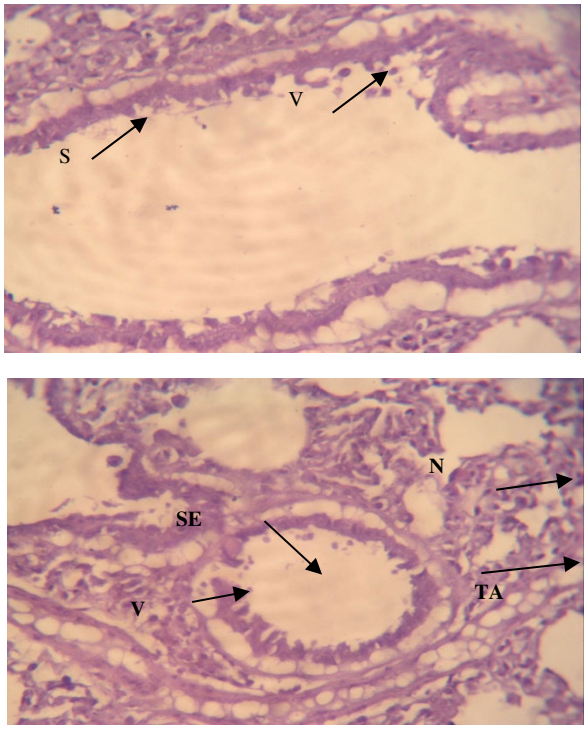

Figure 1: Cross Section of The Lung of The Experimental Group of Mice Treated with A Concentration Of 0.2 Mg/Kg of Pregnyl For 30 Days, Showing the Occurrence of Degenerative Changes in the Bronchioles. Se Separation of Some Epithelial Cells, Ta Thickness of the Alveolar Wall, N Necrosis, V Vacuolation (H and E Stain, 40×)

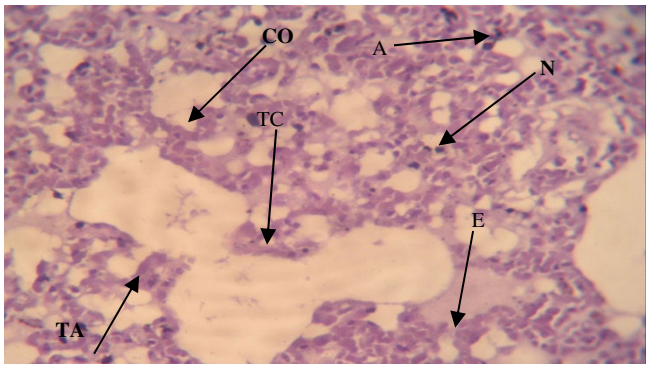

Figure 2: Cross Section of the Lung of the Experimental Group of Mice Treated with A Concentration Of 0.2 Mg/Kg of Pregnyl For 30 Days, Showing the Occurrence of Degenerative Changes in the Lung. Tc Alveolar Duct Wall Thickness, Ta Alveolar Wall Thickness, E Edema, A Infiltration of Inflammatory Cells, Co Vascular Congestion, N Necrosis (H and E Stain, 40×)

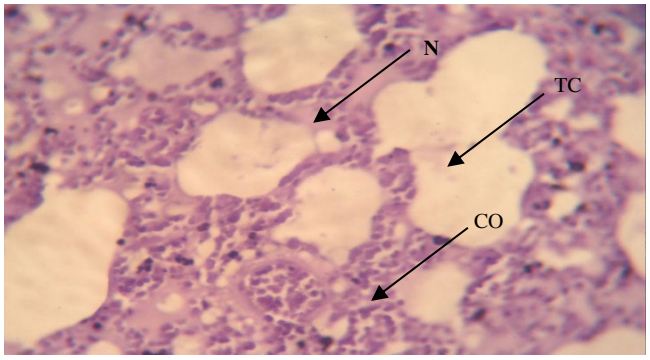

Figure 3: Cross-Section of the Lung of The Experimental Group of Mice Treated with A Concentration Of 0.2 Mg/Kg of Pregnyl For 30 Days, Showing the Occurrence of Congestion in the Blood Vessels of the Lung, N Necrosis in the Alveoli, Co Congestion in the Blood Vessels, Tc Thickness of the Alveolar Duct Wall, (H&E Stain, 40×)

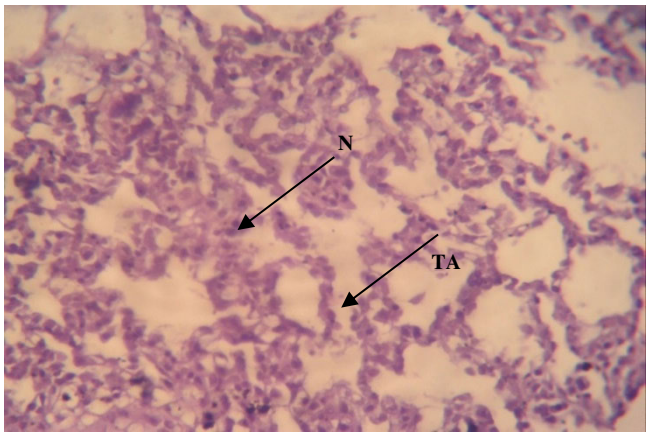

Figure 4: Cross-Section of the Lung of the Experimental Group of Mice Treated with a Concentration of 0.2 Mg/Kg of Pregnyl For 30 Days, Showing the Occurrence of Congestion in the Blood Vessels of the Lung, N Necrosis in the Alveoli, Ta Thickness of the Alveolar Wall, (H&E Stain, 40×)

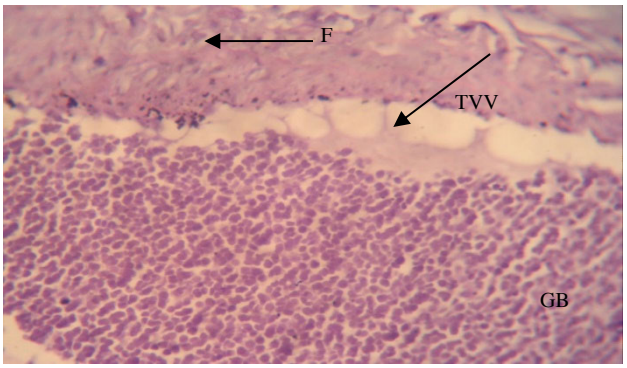

Figure 5: A Cross-Section of the Lung of the Experimental Group of Mice Treated with a Concentration of 0.2 Mg/Kg of Pregnyl For 30 Days, Showing the Occurrence of Congestion in the Blood Vessels of the Lung, a Infiltration of Inflammatory Cells, Co Congestion in the Blood Vessels, Gb Blood Clot, F Fibrosis Tv Active Transport Vesicles. (H&E Stain, 40×)

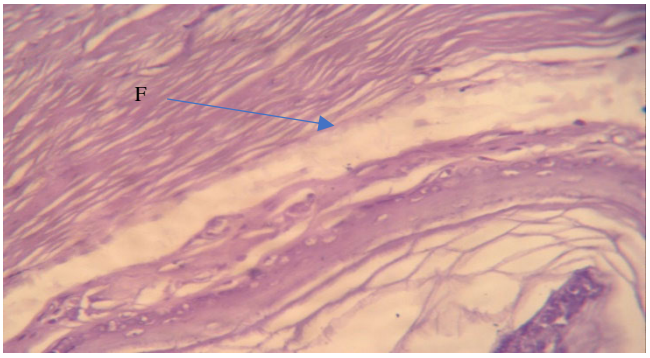

Figure 6: A Cross Section of the Lung of the Experimental Group of Mice Treated with A Concentration of 0.2 Mg/Kg of Pregnyl For 30 Days, Showing the Occurrence of Fibrosis Around the Blood Vessels, F Fibrosis. (H&E Stain, 40×)

Histopathological Changes in Diabetic Rats Treated with 0.2 Mg/Kg of Pregnyl

The results of the histological study of the lungs of mice treated with a concentration of 0.2 mg of pregnyl /kg of body weight for a period of 30 days showed degeneration and necrosis of the lining cells of some bronchi and separation of some cells from the epithelial layer. A gap was also observed between the epithelial tissue and the submucosal layer, as shown in Figure 1.

The results of the study showed the occurrence of edema between the alveoli and alveolar sacs and an increase in the thickness of the wall between the alveoli, as shown in Figure 2.

On the other hand, the results showed congestion and bleeding in the blood vessels, as well as cellular infiltration between the alveoli and alveolar sacs. The results of the study also showed a breakdown in the alveolar wall and alveolar sacs and their accumulation inside the alveolar cavity. It was also noted that inflammatory cells accumulated and that there was congestion between the alveoli and alveolar sacs. Moreover, the results noted that there was a rupture between the alveoli and bleeding, as shown in Figures 3 and 4.

The results showed that the drug pregnyl can affect the lining of the blood vessels inside the organ, and deformation and congestion were observed in the blood vessel with the appearance of a blood clot inside the blood vessel with fibrosis and infiltration of inflammatory cells around the vessel. Active transport vesicles appeared inside the vessels, indicating high activity of the blood vessel lining, as in Figure 5 and 6.

DISCUSSION

The results of the current study showed that the lungs of mice treated with the pregnyl drug at a concentration of 0.2 mg/kg for 30 days suffered from histopathological changes represented by congestion of the blood vessels with the appearance of a blood clot inside them.

The occurrence of vascular congestion may be attributed to excess blood in the venous system, resulting in increased blood pressure in the veins and capillaries. This may exert pressure on adjacent structures and is usually accompanied by decreased blood supply, thus exposing cells to malnutrition, oxygen deficiency, and accumulation of excretory materials [16].

The reason for the appearance of blood clots in the capillaries of the lungs of mice treated with the drug is due to the effect of the drug's chemical substance that led to the precipitation of proteins, which may be the cause of the precipitation of blood proteins. This result is consistent with the study of the researchers [17].

The presence of inflammatory leukocyte infiltration around the blood vessels was a characteristic feature in the tissue sections of the lungs of the mice treated with the drug. This is attributed to the increased effectiveness of the endothelial lining of the blood vessels in transporting inflammatory cells migrating from other parts of the body outside the lung to inside it.

Therefore, the blood vessel appeared vacuolated (containing effective intravascular transport vesicles), which explains the presence of inflammatory cells, the presence of which indicates their important role in defending the body and attempting to carry out cellular repair. This result is consistent with what researchers have reached, including [18].

The inflammatory response is believed to result from degenerative changes in the lung, as the damaged cells secrete inflammatory factors that attract inflammatory cells as a defensive reaction of the body to get rid of the debris of the degenerative cells, which is consistent with the results of the researchers [19].

The results of this study showed that the alveoli and alveolar ducts of the lung were destroyed, and the reason for this was due to the effect of the toxic chemical substance of the drug, as explained by the researchers [17] in their study on the rat, where they indicated that the drug 5-fluorouracil most likely weakened the surface tension of the alveoli due to the insufficient production of surfactant as a result of the cellular damage to the second type of lung cells caused by the drug, and thus leads to the rupture of most of the alveoli, and then continues to spread to the neighboring alveoli, leading to their expansion.

While Giray et al. [20] indicated that such tissue changes could be attributed to a decrease in antioxidants in the animal's body due to the chemical substance of the drug.

Since previous studies indicated that the composition of the alveolar surfaces (in which gas exchange occurs) consists of a protein-lipid complex known as pulmonary surfactant, of which lipids constitute about 95-90% and proteins 5-10%, and the pulmonary surface is formed by type II pneumocytes.

Any deficiency in the components of the pulmonary surface causes a malfunction in its function and structure and can become a life-threatening danger. The pulmonary surface is covered by a membrane known as the bubble film, which covers all areas of the terminal lung unit, thus regulating the distribution of fluids throughout the terminal lung unit. This membrane maintains the surface tension close to zero, thus avoiding the collapse of the pulmonary alveoli [21].

Prengel may have altered the components of the lipid-protein complex that is part of the surface of the alveoli. The increase in the thickness of the alveolar walls in some areas and the decrease in their lumen, which may have come as a result of the expansion of the alveoli and alveolar sacs, made this expansion cause pressure on the alveoli forming the edges of the lung, which led to increased compression and made them characterized by an increase in the thickness of their walls and a decrease in their lumen. This result is consistent with the study of the researchers [19]. The cause of the appearance of edema may be due to the chemical substance of the drug and the increased development of reactions leading to increased oozing of low-protein fluids from the blood vessels, which then collect in the spaces between cells and result in edema [19]. Kumarasinghe and Carroll [22] indicated that edema occurs as a result of increased vascular permeability or salt and water retention,.

On the other hand, pericardium may have an effect on the secretion of the hormone's epinephrine and norepinephrine, which lead to an increase in the branches of the respiratory tree, as we mentioned earlier. It is likely that these branches have led to an increase in pressure on the pulmonary parts, leading to their destruction [23].

The current study also showed an increase in fibrosis in the bronchial epithelium, which may be due to the continuation of inflammatory reactions in these cells, which stimulates the alveolar cells of the second type to divide and transform into fibroblasts by stimulating substances. These fibroblasts begin to produce collagen fibers, which accumulate without differentiating, thus causing pulmonary fibrosis, which appeared in some areas of the damaged tissue in the lungs of mice treated with concentration of the drug Prengel. This result was consistent with the results of the study [17].

REFERENCES

[1] Fournier, T. *et al.* "Review: Hcgs: Different sources of production, different glycoforms and functions." *Placenta,* vol. 36, April 2015, pp. S60-S65. http://dx.doi.org/10.1016/j.placenta.2015.02.002.

[2] Marion, Frédéric *et al.* "Radical cascade cyclizations and platinum(ii)-catalyzed cycloisomerizations of ynamides." *Tetrahedron,* vol. 62, no. 16, April 2006, pp. 3856-3871. http://dx.doi.org/10.1016/j.tet.2005.11.092.

[3] Miller, Walter L. and Himangshu S. Bose. "Early steps in steroidogenesis: Intracellular cholesterol trafficking." *Journal of Lipid Research,* vol. 52, no. 12, December 2011, pp. 2111-2135. http://dx.doi.org/10.1194/jlr.r016675.

[4] Kim, Wan Shin *et al.* "Synthetic nat- or ent-steroids in as few as five chemical steps from epichlorohydrin." *Nature Chemistry,* vol. 10, no. 1, September 2017, pp. 70-77. http://dx.doi.org/10.1038/nchem.2865.

[5] "Foreword." *Modern NMR Approaches to the Structure Elucidation of Natural Products : Data Acquisition and Applications to Compound Classes,* vol. 2, 2016, pp. P005-P007. http://dx.doi.org/10.1039/9781849734684-fp005.

[6] Thao, Nguyen Phuong *et al.* "Steroidal constituents from the edible sea urchin*Diadema savignyi*Michelin induce apoptosis in human cancer cells." *Journal of Medicinal Food,* vol. 18, no. 1, January 2015, pp. 45-53. http://dx.doi.org/10.1089/jmf.2013.3105.

[7] Nwabuobi, Chinedu *et al.* "Hcg: Biological functions and clinical applications." *International Journal of Molecular Sciences,* vol. 18, no. 10, September 2017, pp. 2037-0. http://dx.doi.org/10.3390/ijms18102037.

[8] Bürvenich, Lars *et al.* "Stimulus-induced activation of the glycoprotein hormone α-subunit promoter in human placental choriocarcinoma cells: Major role of a tandem camp response element." *Current Issues in Molecular Biology,* vol. 46, no. 4, April 2024, pp. 3218-3235. http://dx.doi.org/10.3390/cimb46040202.

[9] Nassar G.N. and Leslie S.W. P*hysiology, Testosterone.* StatPearls Publishing, Treasure Island Finland, Jan 2024, https://www.ncbi.nlm.nih.gov/books/NBK526128/

[10] Zirkin, Barry R and Vassilios Papadopoulos. "Leydig cells: Formation, function, and regulation†." *Biology of Reproduction,* vol. 99, no. 1, March 2018, pp. 101-111. http://dx.doi.org/10.1093/biolre/ioy059.

[11] Chen, Fenglei, *et al.* "Ero1α promotes testosterone secretion in hcg-stimulated mouse leydig cells via activation of the pi3k/akt/mtor signaling pathway." *Journal of Cellular Physiology,* vol. 235, no. 7-8, January 2020, pp. 5666-5678. http://dx.doi.org/10.1002/jcp.29498.

[12] Handelsman, David J. *et al.* "Effects of recombinant human lh and hcg on serum and urine lh and androgens in men." *Clinical Endocrinology,* vol. 71, no. 3, August 2009, pp. 417-428. http://dx.doi.org/10.1111/j.1365-2265.2008.03516.x.

[13] Woldemariam, Getachew A. and Anthony W Butch. "Immunoextraction–tandem mass spectrometry method for measuring intact human chorionic gonadotropin, free β-subunit, and β-subunit core fragment in urine." *Clinical Chemistry,* vol. 60, no. 8, August 2014, pp. 1089-1097. http://dx.doi.org/10.1373/clinchem.2014.222703.

[14] Chong, W.C. *et al.* "A Study on Tissue Processing." *IJIR*, no. 3, 2012, pp. 37-43. https://www.researchgate.net/publication/299602601_A_Study_on_Tissue_Processing

[15] Bancroft, J.D. and Gamble, M. *Theory and Practices of Histological Techniques*, 16th Edition, Churchill Livingstone, Elsevier, Philadelphia, 2008, pp. 56. https://www.sciencedirect.com/book/9780443102790/theory-and-practice-of-histological-techniques

[16] Hilscher, Moira, and William Sanchez. "Congestive hepatopathy." *Clinical Liver Disease,* vol. 8, no. 3, September 2016, pp. 68-71. http://dx.doi.org/10.1002/cld.573.

[17] Al-Hamdany, Muna Zuhair, and Abduljabbar Yasin Muhammed Al-Hubaity. "The structural changes of the rat's lung induced by intraperitoneal injection of 5-fluorouracil." *JPMA. The Journal of the Pakistan Medical Association..* vol. 64, no. 7, 2014, pp. 734-738.

[18] Ahmed, Dalal F. "The effect of rifampicin and isoniazid on liver and lung tissues in rats." *Iraqi Journal of Pharmacy,* vol. 11, no. 2, December 2011, pp. 34-45. http://dx.doi.org/10.33899/iphr.2011.49896.

[19] Kumar U. *et al.* "*Robbins Basic pathology*", 7th Edn., Saunders Comp. 2003, pp. 4-781. https://books.google.com.pk/books/about/Robbins_Basic_Pathology_E_Book.html?id=jheBzf17C7YC&redir_esc=y

[20] Giray, Belma *et al.* "Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin e or allopurinol." *Toxicology Letters,* vol. 118, no. 3, January 2001, pp. 139-146. http://dx.doi.org/10.1016/s0378-4274(00)00277-0.

[21] Orgeig, Sandra *et al.* "Recent advances in alveolar biology: Evolution and function of alveolar proteins." *Respiratory Physiology and Neurobiology,* vol. 173, August 2010, pp. S43-S54. http://dx.doi.org/10.1016/j.resp.2010.04.023.

[22] Kumarasinghe, G. and Carroll, G. "A guide to peripheral oedema." *Medicine Today Journal*, vol. 16, no. 6, 2015, pp. 26-34. https://medicinetoday.com.au/mt/2015/june/feature-article/guide-peripheral-oedema

[23] Aronson J.K. *Meyler's Side Effects of Drugs*, 16th Edn. The International Encyclopedia of Adverse Drug Reactions and Interactions, Elsevier, Netherlands, 2016. https://www.sciencedirect.com/referencework/9780444537164/meylers-side-effects-of-drugs#book-description